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Degradation of Benzene, Toluene, Ethylbenzene, and Xylenes (BTEX) by the Lignin-Degrading Basidiomycete *Phanerochaete chrysosporium*

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Degradation of the BTEX (benzene, toluene, ethylbenzene, and *o*-, *m*-, and *p*-xylenes) group of organopollutants by the white-rot fungus *Phanerochaete chrysosporium* was studied. Our results show that the organism efficiently degrades all the BTEX components when these compounds are added either individually or as a composite mixture. Degradation was favored under nonligninolytic culture conditions in malt extract medium, in which extracellular lignin peroxidases (LIPs) and manganese-dependent peroxidases (MNPs) are not produced. The noninvolvement of LIPs and MNPs in BTEX degradation was also evident from in vitro studies using concentrated extracellular fluid containing LIPs and MNPs and from a comparison of the extents of BTEX degradation by the wild type and the *per* mutant, which lacks LIPs and MNPs. A substantially greater extent of degradation of all the BTEX compounds was observed in static than in shaken liquid cultures. Furthermore, the level of degradation was relatively higher at 25 than at 37°C, but pH variations between 4.5 and 7.0 had little effect on the extent of degradation. Studies with uniformly ring-labeled [^{14}C]benzene and [^{14}C]toluene showed substantial mineralization of these compounds to $^{14}CO_2$.

BTEX (benzene, toluene, ethylbenzene, and *o*-, *m*-, and *p*-xylenes) compounds are an important family of organopollutants that are components of gasoline and aviation fuels and are widely used in industrial syntheses (27). BTEX compounds frequently enter soil, sediments, and groundwater because of leakage from underground storage tanks and pipelines, accidental spills, improper waste disposal practices, and leaching landfills (6, 11, 26, 32). The BTEX compounds are carcinogenic and neurotoxic (8) and are classified as priority pollutants regulated by the Environmental Protection Agency (31). Both aerobic and anaerobic bacteria have been shown to degrade BTEX compounds (9, 10, 12, 17, 27), but most of these studies on bacterial degradation of BTEX have used microbial consortia and no pure strain of a bacterium is known to degrade all the components of BTEX efficiently. Particularly, *o*-xylene has been known to be recalcitrant to bacterial degradation (10). Attempts to genetically modify bacteria to increase the degradation range of a single organism are being made (14, 25); however, there is still some controversy regarding the release of genetically engineered microorganisms into the environment. Furthermore, problems due to inhibitory interactions among different components of BTEX during bacterial degradation of BTEX mixtures have been reported (1, 2). Since the BTEX compounds are known to occur as a mixture in contaminated sites, an organism that simultaneously degrades all the different components of BTEX is more desirable than an organism that degrades only some of the BTEX compounds (14).

Recent studies with *Phanerochaete chrysosporium*, a naturally occurring wood-degrading white-rot fungus, have shown that it not only mineralizes lignin and related compounds (5, 20) but also is versatile in its ability to degrade a wide spectrum of recalcitrant organopollutants, such as chlorinated phenols, polychlorinated biphenyls, chlorinated dibenzodioxins, alkylhalide insecticides, nitroaromatics, polynuclear aromatic hydrocarbons, and kraft bleach plant effluents (3, 15, 22, 23, 33–35). In many of these reports, biodegradation has been observed primarily under ligninolytic conditions and was associated with two key families of extracellular peroxidases, designated lignin peroxidases (LIPs) and manganese-dependent peroxidases (MNPs). Furthermore, biodegradation of the organopollutants was observed only during secondary metabolism induced by starvation for nutrient nitrogen or carbon. However, degradation of the BTEX family of organopollutants by *P. chrysosporium* had not been reported to date. In this paper, we report for the first time that *P. chrysosporium* simultaneously degrades all the BTEX components and that this degradation is favored under nonligninolytic culture conditions when no LIPs and MNPs are produced.

MATERIALS AND METHODS

Strains. *P. chrysosporium* ME-446 (ATCC 34541) and BKM-F 1767 (ATCC 24725) were maintained by subculturing on 2% malt extract agar slants, pH 4.5 (18). The peroxidase-negative (*per*) mutant (19, 22) used in some of the experiments was derived from strain ME-446.

Chemicals. Benzene, toluene, ethylbenzene, and the *o*-, *m*-, and *p*- isomers of xylene were purchased from Aldrich Chemical Co. (Milwaukee, Wis.), and each had >99.9% purity. Aqueous stock solutions for each of these compounds were prepared in serum bottles (Wheaton, Millville, N.J.) sealed with Teflon-coated rubber stoppers and aluminum crimps. *m*-Toluic acid (99% pure) and *p*-toluic acid (98% pure), obtained from the same source, were converted to their potassium salts by dissolving in distilled water and neutralizing to pH 7.0 with 1 M KOH.

Radiochemicals. Uniformly ring-labeled [^{14}C]benzene

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(57.5 mCi/mmol; >98% radiochemically pure) and [^{14}C]toluene (10.2 mCi/mmol; >98% radiochemically pure) were purchased from Sigma Chemical Co. (St. Louis, Mo.). The chemical purities of [^{14}C]benzene and [^{14}C]toluene were 99.9 and 98.6%, respectively, on the basis of gas chromatography (GC) analyses provided by the manufacturer. The radiochemicals were stored as ethanolic stock solutions.

Media. Three types of liquid media were used in this study. A defined low-nitrogen basal III medium (low-N medium) contained 1% glucose, 2.4 mM N as ammonium tartrate, minerals, thiamine, veratryl alcohol, Tween 80, and 20 mM sodium acetate buffer (pH 4.5) (7). High-N medium had the same composition as the low-N medium, except that it had a 10-fold-higher nitrogen content. Malt extract medium (ME medium) contained 2% malt extract (Difco Laboratories, Detroit, Mich.), 2% glucose, and 0.1% Bacto Peptone, and the pH was adjusted to 4.5.

Inoculum. An aqueous suspension of conidia of a given fungal strain was prepared from 5-day-old malt extract agar plates incubated at 37°C (4). A 50-ml volume of low-N medium (without Tween 80) in a sterile 3-liter Fernbach flask was inoculated with the conidial suspension (approximately 8×10^6 conidia per flask) and incubated at 37°C for 48 h under static conditions. The culture was blended aseptically for 5 min at setting 5 in a Sorvall Omni-mixer (model 17150; Ivan Sorvall Inc., Newtown, Conn.), and this blended mycelial inoculum was used at the 10% (vol/vol) level for both the shaken and the static cultures described below.

Culture conditions. In BTEX degradation experiments, the fungus was grown as either shaken (50-ml volume; 200 rpm) or static (10-ml volume) liquid cultures in 125-ml serum bottles (Wheaton) sealed with Teflon-coated grey butyl rubber stoppers (The West Co., Phoenixville, Pa.) and aluminum crimps (Baxter Scientific Products, McGaw Park, Ill.). Each of the inoculated bottles was oxygenated for 1 min before being spiked with the appropriate BTEX compound(s). Each of the BTEX compounds was added at a concentration of 10 mg/liter (10 ppm) except where mentioned otherwise. The cultures were incubated at 37 or 25°C as indicated. Three replicates were used for each treatment. Uninoculated medium controls and heat-killed culture controls (each in duplicate) were also included for each treatment. Heat-killed controls consisted of cultures that were pregrown for 7 days under conditions identical to those of the corresponding experimental cultures and then killed by autoclaving. The fungal biomass of the heat-killed controls was approximately equivalent to that in the experimental cultures. In time course studies, the appropriate sample and control bottles were sacrificed at each time point for analysis. Since toluates are nonvolatile, their degradation was studied with 50-ml liquid cultures in 125-ml Erlenmeyer flasks sealed with rubber stoppers and incubated at 37°C at 200 rpm. These cultures were oxygenated daily during incubation. The cultures were routinely examined by phase-contrast microscopy to ensure the absence of bacterial contamination.

For determining the extent of degradation by mycelial pellets versus extracellular peroxidases, washed mycelial pellets (0.5 g) from 6-day-old cultures grown in low-N medium were added to 2 ml of reaction mixture containing 10 mM sodium acetate buffer (pH 4.5), 10 μg of ethylbenzene, and 20 mM glucose. The reaction mixture, in 10-ml sealed serum vials, was incubated at 37°C for 24 h. A parallel control with heat-killed pellets was used for comparison. Extracellular culture fluid from the same cultures was concentrated 50-fold as described elsewhere (4) and was used as a source of LIPs (LIP activity, 8,000 U/liter). The reaction mixture contained (in 2 ml) 8 U of LIP, 10 μg of ethylbenzene, 0.2 mM H_2O_2, and 20 mM sodium tartrate buffer, pH 3.0. The mixture was incubated at 37°C for 24 h.

GC. The concentration of BTEX compounds was measured by GC analysis of the headspace. All samples, irrespective of the incubation temperature of the cultures, were equilibrated at 25°C before GC analysis. A 500-μl headspace sample, drawn with a 1-ml Pressure-Lok gas-tight syringe, was injected into the gas chromatograph (Varian series 3700) equipped with a DB-624 fused-silica megabore column (30 m [length] by 0.53 mm [inside diameter]; film thickness, 3.0 μm) (J & W Scientific, Folsom, Calif.) and a flame ionization detector attached to a Hewlett-Packard integrator. The operating conditions were as follows: column temperature, 90°C; injector and detector temperature, 200°C; carrier gas, He; and total flow rate, 15 ml/min. The column temperature was lowered to 45°C to resolve ethylbenzene and xylenes when the BTEX mixture was analyzed.

The oxygen concentration in the culture was monitored by GC analyses of the headspace with a Gow-Mac (Bridgewater, N.J.) model 350 gas chromatograph equipped with a CTR column (Alltech Associates, Deerfield, Ill.) and a thermal conductivity detector. The operating conditions were as follows: column temperature, ambient; injector and detector temperature, ambient; carrier gas, He; flow rate, 65 ml/min; and bridge current, 200 mA.

HPLC. The toluate concentration in shaken cultures was monitored by high-pressure liquid chromatography (HPLC) analysis of liquid-phase samples (filtered through a Millipore Millex-GS syringe filter; 0.45-μm pore size) with a Hewlett-Packard series 1050 HPLC equipped with a Lichrosorb RP-18 column (Anspec Co., Ann Arbor, Mich.) and a UV detector set at 230 nm, with methanol–0.1% phosphoric acid (60:40) used as the eluant.

Other analyses. LIP and MNP activities were, respectively, estimated by the procedures of Tien and Kirk (30) and Paszczynski et al. (24). Fungal biomass was measured as mycelial dry weight as previously described (22).

Mineralization experiments. Appropriate amounts of ^{14}C-labeled benzene or toluene were added to cultures right after inoculation, along with a 5-mg/liter concentration of the respective unlabeled compound. At specified intervals during the incubation, the $^{14}CO_2$ evolved in each bottle was flushed out with CO_2-free air and was trapped in a two-stage trapping system consisting of two successive 1 N NaOH traps of 10 ml each in serum vials. One-milliliter aliquots of the traps were taken in glass liquid scintillation vials, purged with CO_2-free air to remove any free volatiles, and then mixed with 15 ml of scintillation cocktail (Safety Solve; Research Products International Corp., Mount Prospect, Ill.), and counting was done by using a model 6892 liquid scintillation system (Tracor Analytic, Elk Grove Village, Ill.). Counting efficiency was monitored by using an external standard. On the basis of data from a number of experiments, 92 to 98% of the $^{14}CO_2$ (as determined by counting) was trapped in the first NaOH trap while the rest was trapped in the second NaOH trap. In independent experiments, the accuracy of the $^{14}CO_2$ measurements obtained by the NaOH trapping system described above was verified by using a barium chloride precipitation step (13). On the basis of these data, the mean barium chloride nonprecipitable disintegrations per minute in the first NaOH trap were 1.1%, whereas in the second NaOH trap, none were detectable.

TABLE 1. Degradation of BTEX compounds and toluates in different media by *P. chrysosporium*[a]

Compound	% Degradation[b] under the indicated conditions			
	Low-N medium, 37°C	High-N medium, 37°C	ME medium	
			37°C	25°C
Benzene	2.2 ± 1.7	0.6 ± 0.4	5.0 ± 2.9	13.2 ± 1.4
Toluene	22.4 ± 1.3	9.3 ± 3.2	31.3 ± 2.9	70.9 ± 2.9
Ethylbenzene	48.3 ± 4.1	27.7 ± 2.3	84.0 ± 1.0	89.0 ± 1.5
o-Xylene	16.7 ± 1.8	14.6 ± 2.1	32.0 ± 2.6	56.0 ± 1.6
m-Xylene	11.2 ± 1.4	6.7 ± 2.6	40.0 ± 5.2	57.1 ± 0.5
p-Xylene	21.6 ± 1.6	7.5 ± 3.8	53.5 ± 8.2	60.0 ± 6.0
m-Toluate	99.3 ± 0.3	100 ± 0.0	NT	NT
p-Toluate	100 ± 0.0	100 ± 0.0	NT	NT

[a] *P. chrysosporium* ME-446 was grown as shaken cultures (50 ml) in sealed 125-ml serum bottles in defined low-N medium (2.4 mM N), high-N medium (24 mM N), or ME medium (8 mM total N) as described in Materials and Methods. Each bottle was inoculated with blended mycelial inoculum (10%, vol/vol), sparged with 100% oxygen, and sealed with a Teflon-coated grey butyl rubber stopper and aluminum crimp. The BTEX compounds were added at a concentration of 10 mg/liter except for toluates, which were each added at a concentration of 68 mg/liter. The bottles were shaken at 200 rpm at 37 or 25°C for 5 days.

[b] All values were corrected for the sorption values obtained with the heat-killed controls. Values represent means ± standard deviations for three replicates. NT, not tested.

RESULTS

Degradation of BTEX compounds. Our results showed degradation of the BTEX compounds by *P. chrysosporium* in the ME medium as well as in the high-N medium, in which no LIP and MNP production was seen (data not shown). Furthermore, the extent of degradation in ME medium was much greater than that observed in the low-N medium (Table 1), in which there was full expression of LIPs and MNPs. Extensive degradation of *m*- and *p*- toluates was observed in both high-N and low-N media (Table 1). Moreover, this degradation occurred within the first 24 h of incubation, during which time LIPs and MNPs are not known to be produced (22).

We then investigated BTEX degradation by concentrated extracellular culture fluid versus washed mycelial pellets and by wild-type *P. chrysosporium* (ME-446) versus its peroxidase-negative mutant (*per*). Ethylbenzene was used as the substrate model in these studies. About 41.4% of the added ethylbenzene disappeared in the presence of *P. chrysosporium* pellets, while little, if any, degradation occurred in the presence of concentrated extracellular fluid containing LIPs and MNPs. Ethylbenzene degradation by the *per* mutant and wild-type *P. chrysosporium* (Table 2) further supported the conclusion that LIPs and MNPs are not involved in ethylbenzene degradation.

Most of the reports to date on degradation of organopollutants by *P. chrysosporium* have been based on growth of the organism at 37°C (3, 15), and there have been relatively few studies on degradation of these pollutants at ambient temperature, which is obviously more relevant to bioremediation applications. We observed substantially greater degradation of all the BTEX compounds (except ethylbenzene) at 25 than at 37°C (Table 1). For example, toluene and *o*-xylene degradation at 25°C was almost twice as much as that observed at 37°C. Further results showed no appreciable differences in toluene degradation in the pH range 4.5 to 7.0 (data not shown). Oxygenation of the cultures gave an over-twofold increase in degradation compared with that of unoxygenated but otherwise identical controls. As various

TABLE 2. Degradation of ethylbenzene by washed mycelial pellets, extracellular culture fluid, whole cultures, and a *per* mutant of *P. chrysosporium*

Treatment	% Degradation[a]
Mycelial pellets[b]	41.4 ± 0.9
Extracellular fluid[b]	4.0 ± 3.9
Whole culture[c]	52.5 ± 1.7
per mutant[c]	50.5 ± 3.0

[a] Values represent means ± standard deviations for three replicates. All values shown are net values corrected for the values for the corresponding heat-killed controls.

[b] Preparation of the mycelial pellets and 50×-concentrated extracellular culture fluid and the reaction conditions are described in Materials and Methods. Incubation was at 37°C for 24 h.

[c] Whole cultures of ME-446 and its *per* mutant were grown in low-N medium (containing 10 mg of ethylbenzene per liter) for 6 days at 37°C. All other culture conditions were as described in Table 1, footnote *a*.

levels of BTEX compounds have been reported at different contamination sites (0.2 to 10.0 mg/liter), we studied the effect of concentration on the extent of degradation. Degradation was seen with initial toluene concentrations of 0.25 to 20 mg/liter, although the percentage of degradation was lower at higher concentrations. However, the same was not true in the case of ethylbenzene (Table 3), which was degraded at a high level at all concentrations tested.

Kinetics of degradation. Most of the degradation of toluene and *o*-, *m*-, and *p*-xylenes was observed in the first 4 to 5 days (after which the rate slowed down), while most of the ethylbenzene disappeared in 2 days (Fig. 1). Moreover, the rates of disappearance of toluene, ethylbenzene, and xylenes essentially paralleled growth rates (measured as the increase in dry weight of the fungal biomass). The order of degradation of BTEX compounds was ethylbenzene > toluene > xylenes > benzene (Table 1).

Static versus shaken culture. The extent of degradation of BTEX compounds in static cultures was substantially greater than that in shaken cultures (Fig. 1 and 2). For example, 34.2% of the benzene disappeared in static cultures, compared with 22.2% in shaken cultures. However, ethylbenzene was degraded almost completely in both static and shaken cultures. The corresponding values for the other BTEX compounds were in the ranges 86 to 95 and 65 to 70% for static and shaken cultures, respectively. Unlike in shaken cultures, the degradation in static cultures continued to increase after the first week of incubation for all the

TABLE 3. Effects of initial concentrations of toluene and ethylbenzene on degradation[a]

Initial concn (mg/liter)	% Degradation	
	Toluene	Ethylbenzene
0.25	80.0 ± 3.7	92.0 ± 1.2
0.50	61.0 ± 4.5	96.0 ± 0.0
1.0	57.0 ± 2.6	98.0 ± 0.7
2.0	50.0 ± 3.0	98.5 ± 0.01
5.0	45.5 ± 2.1	99.4 ± 0.2
10.0	40.4 ± 4.2	98.0 ± 2.2
20.0	29.5 ± 0.5	90.5 ± 3.2

[a] Cultures were grown in ME medium in oxygenated, sealed serum bottles at 37°C for 5 days. Other culture conditions were as described in Table 1, footnote *a*. Values represent means ± standard deviations for triplicate cultures.

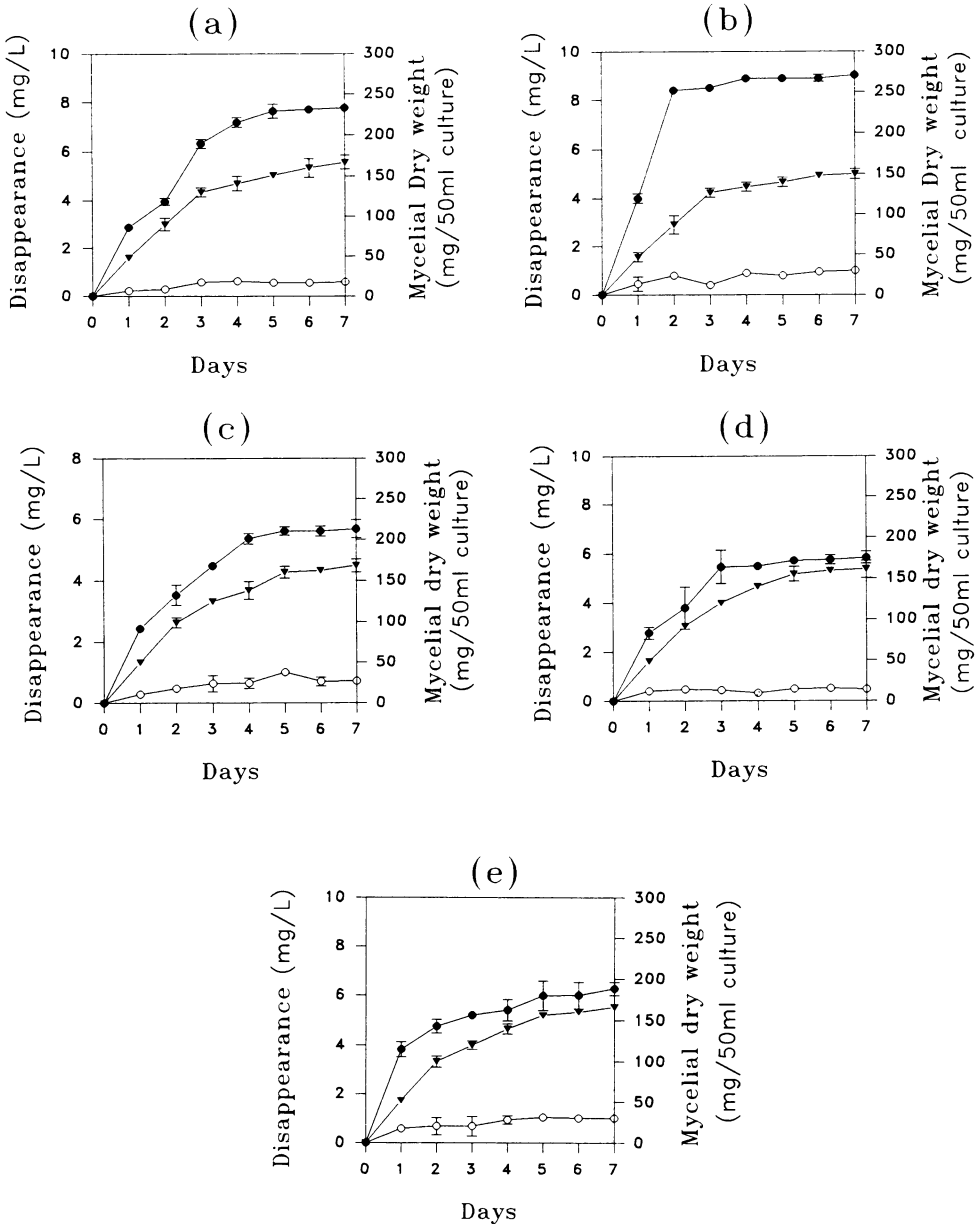

FIG. 1. Time course of degradation of toluene (a), ethylbenzene (b), *o*-xylene (c), *m*-xylene (d), and *p*-xylene (e) in shaken cultures of *P. chrysosporium*. Cultures were grown in ME medium at 25°C. Each compound was added to give a final concentration of 10 mg/liter. Other culture conditions were as described in Table 1, footnote *a*. Mycelial dry weights are also presented for comparison. Mycelial dry weights of the heat-killed controls (prepared as described in Materials and Methods) ranged from 150 to 172 mg/50-ml culture. Values plotted are means ± standard deviations for triplicate cultures. Symbols: ○, heat-killed control; ●, experimental culture; ▼, mycelial dry weight.

compounds except benzene, which showed continued degradation in both shaken and static cultures.

Degradation of BTEX mixture. Since BTEX compounds are present as a composite mixture and not as single compounds in naturally occurring contamination sites, the rate and extent of degradation of such a composite mixture were studied. The results indicated that all components of the BTEX mixture are simultaneously degraded by *P. chrysosporium* (Fig. 3). Furthermore, the extent of degradation of different components of the mixture was comparable to that observed when these compounds were tested singly (except for toluene). Also, the total extent of degradation of the BTEX mixture was greater at 25 than at 37°C (data not shown). Most of the degradation of the BTEX mixture occurred in the first week of incubation, and no further increase in degradation was noticed in the subsequent 2 weeks. The extents of degradation of various BTEX compounds by ME-446 and BKM-F 1767, the two most widely studied strains of *P. chrysosporium*, were comparable, although BKM-F 1767 tended to show higher levels of benzene degradation and lower levels of *m*- and *p*-xylene degradation than ME-446. Furthermore, ME-446 and its peroxidase-negative mutant gave comparable levels of degradation of the BTEX mixture (Table 4), which is consistent with the

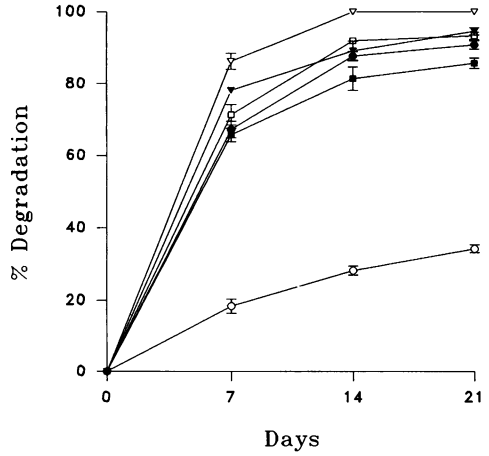

FIG. 2. Degradation of BTEX compounds in static cultures of *P. chrysosporium*. The cultures (10 ml) were grown in ME medium in 125-ml sealed serum bottles at 25°C as described in Materials and Methods. Each compound was added to give a final concentration of 5 mg/liter. Values plotted are means ± standard deviations for triplicate cultures. Symbols: ○, benzene; ●, toluene; ▽, ethylbenzene; ▼, *o*-xylene; □, *m*-xylene; ■, *p*-xylene.

earlier conclusions that LIPs and MNPs are not important in BTEX degradation. However, the reason for the substantially lower level of degradation of ethylbenzene by the *per* mutant compared with that by ME-446 is not clear.

Mineralization. Considering the relatively low rate of degradation of benzene, based on the GC data presented above, we studied the mineralization of uniformly ring-labeled [^{14}C]benzene. Our results showed 12.3% mineralization of benzene in a 2-week period. The corresponding figure for percent disappearance of unlabeled benzene during the same 2-week period was about 30% (Fig. 2). The extent of mineralization of uniformly ring-labeled [^{14}C]toluene in a parallel experiment was about 50% (Fig. 4). These results indicate that *P. chrysosporium* mineralizes benzene, albeit to a lesser extent than toluene. The extent of mineralization of both benzene and toluene was much less in shaken cultures than in static cultures (data not shown).

TABLE 4. Comparative degradation of BTEX mixture by *P. chrysosporium* ME-446 and BKM-F 1767 and a peroxidase-negative (*per*) mutant of ME-446

Components of BTEX mixture[a]	% Degradation[b] by:		
	ME-446	BKM-F 1767	*per* mutant
Benzene	9.6 ± 1.8	18.1 ± 2.8	14.1 ± 3.0
Toluene	35.8 ± 2.9	41.4 ± 1.0	36.5 ± 0.7
Ethylbenzene	93.4 ± 1.6	99.5 ± 0.4	79.6 ± 0.5
o-Xylene	48.7 ± 2.6	44.8 ± 3.1	49.4 ± 1.1
m-Xylene, *p*-xylene[c]	63.2 ± 2.6	52.3 ± 3.4	67.2 ± 0.6

[a] BTEX mixture (which contained 3 mg of each of the listed components per liter) was added to the ME medium cultures. Incubation was at 25°C for 5 days. Other culture conditions were as described in Table 1, footnote *a*. Percent degradation of each of the components in the mixture was measured at the end of the incubation period.

[b] Values represent means ± standard deviations of triplicate cultures.

[c] *meta* and *para* isomers of xylene were separated as a single peak in the gas chromatogram.

DISCUSSION

The results of this study demonstrate extensive degradation of all the BTEX compounds as well as the toluates by *P. chrysosporium*. The idea that the observed degradation is due to fungal metabolism and not due to sorption to mycelial biomass is based on several lines of evidence. First, the data on the extent of disappearance of toluene, ethylbenzene, and the xylenes in experimental cultures versus heat-killed controls (Fig. 1) clearly support the idea that the observed disappearance is due to fungal metabolism. Second, the high-N medium and the ME medium gave comparable fungal

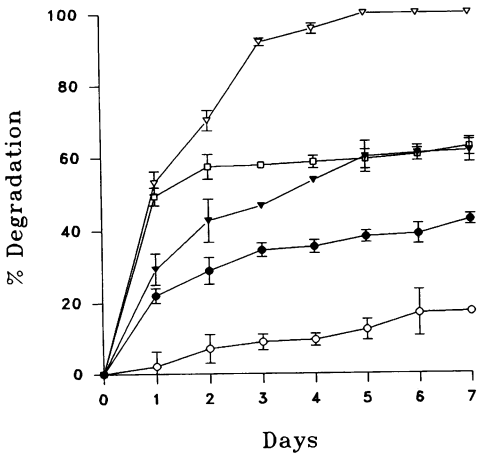

FIG. 3. Degradation of a mixture of BTEX compounds (3 mg of each component per liter) in shaken cultures of *P. chrysosporium*. Culture conditions were as described in the legend to Fig. 1. Values plotted are means ± standard deviations for triplicate cultures. Symbols: ○, benzene; ●, toluene; ▽, ethylbenzene; ▼, *o*-xylene; □, *m*- and *p*-xylene. The *meta* and *para* isomers of xylene appeared as a single peak when the BTEX mixture was analyzed by GC.

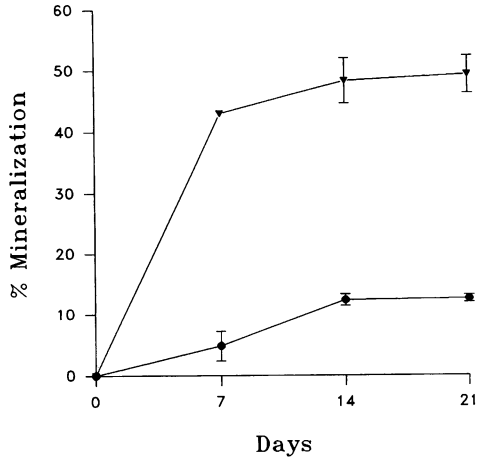

FIG. 4. Mineralization of uniformly ring-labeled [^{14}C]benzene (●) and [^{14}C]toluene (▼) by *P. chrysosporium*. Cultures (10 ml) were grown under static conditions as described for Fig. 2. Each culture contained 0.5 × 10^6 cpm of the radioactive compound and 5 mg of the cold compound per liter to obtain final specific activities of 0.484 ([^{14}C]benzene) and 0.57 ([^{14}C]toluene) mCi/mmol. The radiochemical purities of both [^{14}C]benzene and [^{14}C]toluene were >98%. Values plotted are means ± standard deviations for triplicate cultures.

biomasses (data not shown) but gave very different levels of degradation (Table 1). Finally, the net mineralization values reported in Fig. 4 also indicate that the observed degradation is due to fungal metabolism.

Our data show that *P. chrysosporium* rapidly degrades BTEX compounds in ME medium, in which LIP and MNP production as well as the lignin degradation system as a whole is suppressed (Table 1). Likewise, Spiker et al. (28) have recently shown that trinitrotoluene is degraded in malt extract broth by this organism. The results of the in vitro experiment (Table 2) using ethylbenzene as the substrate further indicate that the degradation activity resides in the mycelial pellets and that extracellular peroxidases in the culture fluid are not involved in this process. Moreover, comparable levels of degradation of BTEX compounds observed with the *per* mutant, which lacks the ability to produce LIPs and MNPs, and the wild-type parent strain (ME-446) are consistent with the idea that extracellular peroxidases are not involved in the degradation of BTEX compounds (Tables 2 and 4). These data are consistent with similar reports on the noninvolvement of LIPs and MNPs in the degradation of certain other organopollutants (16, 21, 29, 35). Finally, on the basis of their ionization potential, the BTEX compounds are unlikely to be the substrates for LIPs. For example, the ionization potentials of benzene (9.24 eV), toluene (8.82 eV), *o*-xylene (8.56 eV), *m*-xylene (8.58 eV), and *p*-xylene (8.44 eV) are much higher than that required for LIP activity ($\leq$7.55 eV), as reported in earlier work (15).

Physiological parameters appear to strongly affect the degradation of BTEX compounds by *P. chrysosporium*, as indicated by stimulation of degradation of toluene by oxygenation. Since we used in the present study a closed culture system which limited the supply of oxygen, the cessation of degradation within a week in shaken cultures (50 ml of medium per 125-ml flask) could probably be due to the exhaustion of available oxygen in the headspace. In fact, our GC measurements indicated at least 95.4% available oxygen consumption in 5 days in these cultures. The higher and sustained levels of degradation of BTEX compounds observed in shallow static cultures (10 ml of medium per 125-ml flask), which had greater headspace volume and hence more available oxygen, are consistent with this idea (Fig. 2). Enhanced degradation at ambient temperature (25°C) and no adverse effect of neutral pH on degradation are added advantages for potential field application of this fungus in the bioremediation of contaminated materials and sites.

Substantial disappearance of most of the BTEX compounds within the first 2 days of incubation suggests that the BTEX degradation system is expressed during primary metabolism and there appears to be no lag period involved. This is in contrast to the lignin degradation process, which occurs during secondary metabolism and is preceded by a lag period of about 3 to 4 days (5).

P. chrysosporium degraded all the BTEX components either individually or as a composite mixture (Fig. 3), suggesting a lack of inhibitory interactions among these components. However, toluene was degraded to a relatively lesser extent in the mixture than when it was tested alone or in combination with benzene. These data suggest that the fungus has a preference for compounds with higher substituted aromatic rings, such as ethylbenzene and xylenes, over toluene.

Our results indicate that *P. chrysosporium* mineralizes benzene and toluene to CO_2 (Fig. 4). Comparison of the extent of mineralization of benzene and toluene with their total disappearance values, based on GC analysis (Fig. 2), indicates that about half of the degradation is in the form of mineralization. This partial mineralization of the compounds, compared with disappearance, could possibly be due to involvement of one or more rate-limiting steps in the degradative pathway, slow turnover of the degradative enzyme(s), and/or accumulation of inhibitory metabolic intermediate(s). Further studies on characterization of the intermediary metabolites produced during the degradation of various BTEX compounds and elucidation of the degradation pathways should provide answers to some of these questions.

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